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ENZYMES AND OTHER AGENTS THAT ENHANCE CELL WALL EXTENSIBILITY

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ABSTRACT

Polysaccharides and proteins are secreted to the inner surface of the growing cell wall, where they assemble into a network that is mechanically strong, yet remains extensible until the cells cease growth. This review focuses on the agents that directly or indirectly enhance the extensibility properties of growing walls. The properties of expansins, endoglucanases, and xyloglucan transglycosylases are reviewed and their postulated roles in modulating wall extensibility are evaluated. A summary model for wall extension is presented, in which expansin is a primary agent of wall extension, whereas endoglucanases, xyloglucan endotransglycosylase, and other enzymes that alter wall structure act secondarily to modulate expansin action.

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INTRODUCTION

From an assemblage of structurally diverse polysaccharides, proteins, phenolic compounds, and other materials, plant cells fashion a complex wall that serves many functions. These include structural support and cell shape; protection against pathogens, dehydration, and other environmental assaults; storage and release of signaling molecules; and storage of carbohydrates, metal ions, and other materials. In the formative phase of a cell's life, the wall plays a determinative role in establishing the size and shape of the cell. To grow, plant cells selectively extend their cell walls, resulting in larger cells and modified cell shapes. Each of the approximately two dozen cell types that comprise the plant body are recognizable, to a large extent, by their distinctive size, shape, and wall morphology—characteristics that emerge from the history of wall growth. Thus, plant cell growth and differentiation inevitably depend upon precise spatial and temporal patterns of wall growth, patterns that are uniquely regulated in each cell type.

The fundamental structure of the primary (growing) cell wall in all land plants appears to be very similar: cellulose microfibrils embedded in a hydrated matrix composed mostly of neutral and acidic polysaccharides and a small amount of structural proteins. Recent reviews describe the structure and function of wall polysaccharides (13) and proteins (14). In this review I discuss recent results dealing with the molecular control of wall extensibility. Other recent reviews deal with polysaccharide-modifying enzymes with other points of focus (42, 85, 86).

THE NOTION OF WALL "LOOSENING"

The concept that the growing cell wall must be "loosened" in order to expand its surface arises from various biophysical, biochemical, and physiological considerations (see 13, 24, 41). The growing wall behaves like a network of inextensible cellulose microfibrils laterally linked together via a complex matrix of flexible polysaccharides that may bind to cellulose and to each other. Current models of the wall envision hemicelluloses, such as xyloglucan, coating the surface of cellulose and directly linking microfibrils together (Figure 1). Such crosslinks have been observed microscopically (73), but whether there are enough of them to contribute substantially to wall strength is uncertain. An alternative model [e.g. (110)] that is equally consistent with experimental data shows microfibrils coated with hemicelluloses and embedded in additional layers of matrix polymers, that is, without direct

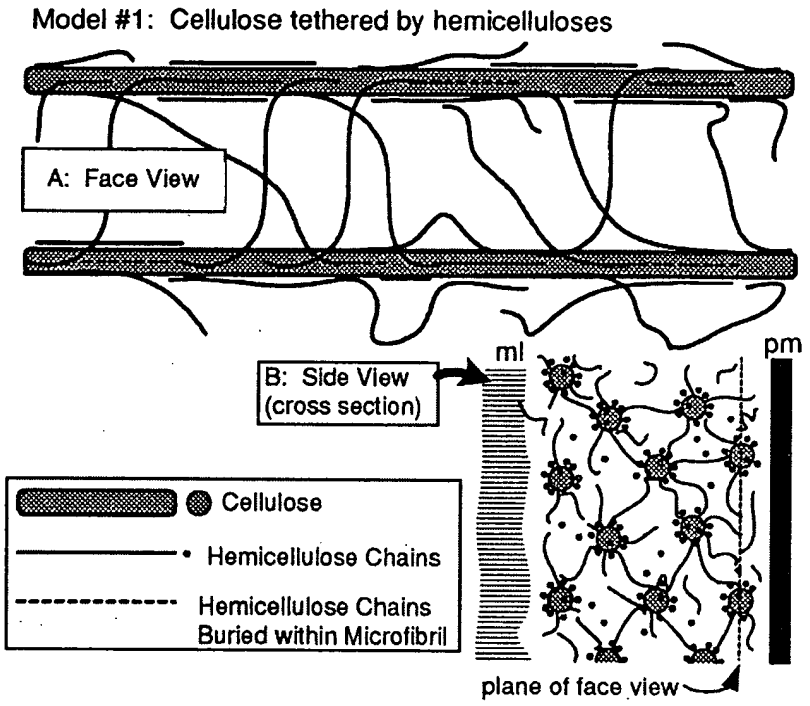

Figure 1 A model (#1) of the growing plant cell wall. *A*: View in the plane of the wall (face view, parallel to the plasma membrane). *B*: Side view, at right angle to *A*, giving a cross section of the wall. The plane of the face view in *A* is indicated by a dotted line. ml = middle lamella; pm = plasma membrane. This model attributes the mechanical strength of the wall to tethering of cellulose microfibrils by xyloglucans that are bonded noncovalently to the microfibril surface and entrapped within the microfibril. Pectins (not shown) are viewed as forming a co-extensive matrix in which the cellulose-xyloglucan network is embedded. This picture of the wall is found in many current discussions of cell walls, e.g. (13, 24, 41, 48, 72, 86).

microfibril-microfibril links (Figure 2). In this model, wall strength may depend largely on many noncovalent interactions between the laterally aligned matrix polymers.

Regardless of which picture of the wall is closer to reality, this microfibril/matrix network is strong enough to resist the high tensile forces generated within the wall as it resists the outward force of cell turgor pressure. Wall enlargement requires controlled spreading of the cellulose/matrix network, evidently as a result of rearrangement of the matrix polymers (23). As a viscoelastic material, cell walls have inherent extension and yield properties that stem from the basic structure of the wall, and some attempts have been made to account for cell wall extension purely in terms of these viscoelastic properties (117, 123). However,

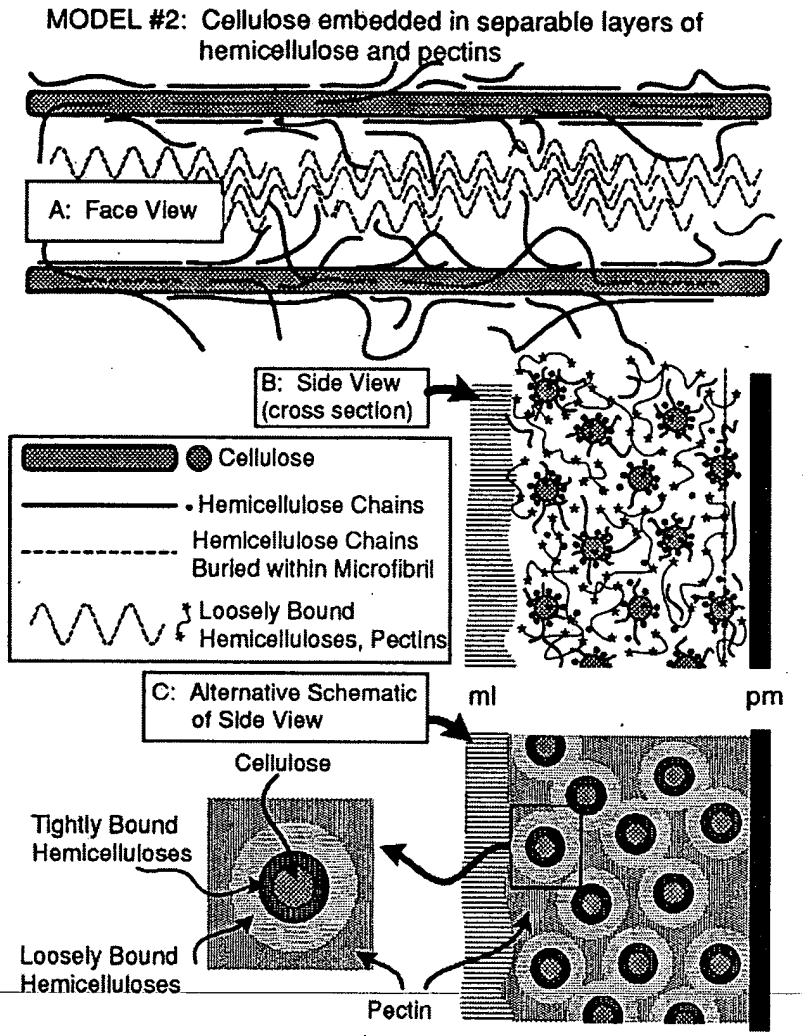

Figure 2 An alternative model (#2) of the growing plant cell wall. *A*: View in the plane of the wall (face view, parallel to the plasma membrane). *B*: Side view, at right angle to *A*, giving a cross section of the wall. The plane of the face view in *A* is indicated by a dotted line. *C*: An alternative depiction of the side view, showing more clearly the different "layers" in which the microfibrils are thought to be coated and embedded. This model differs from that in Figure 1 primarily by the lack of polymers that directly cross-link the microfibrils. Instead, the tightly bound hemicelluloses, such as xyloglucan, are pictured as sheathed in a layer of less tightly bound polysaccharides, which in turn are embedded in the pectin matrix that fills the spaces between the microfibrils. This model is adapted from Talbott & Ray (110), who cite older literature supporting similar models. ml = middle lamella; pm = plasma membrane.

simple wall viscoelasticity appears to be insufficient to account for the extension behaviors of growing walls (23). For instance, isolated walls treated so as to inactivate wall enzymes extend only transiently when put in tension. To a first approximation, such inactivated walls act like a viscoelastic solid. In contrast, the walls of growing cells exhibit a steady, long-term creep (a type of irreversible extension), and such creep may be mimicked in isolated walls, as long as their associated proteins are not inactivated (21, 22). Thus, protein-mediated loosening processes, perhaps in combination with integration of newly secreted polymers, seem to be required to catalyze and sustain anything more than a limited viscoelastic extension of the wall.

Several types of polymer rearrangements could plausibly lead to turgor-driven wall expansion. These include weakening of the noncovalent bonding between polysaccharides (as postulated for expansins), cleavage of the backbone of the major matrix polymers (e.g. by endoglucanases, pectinases, transglycosylases, and hydroxyl radicals), and breakage of crosslinks between matrix polymers (e.g. by esterases). It is a curious fact that the only agent (expansin) shown to catalyze wall extension in vitro acts by a still mysterious biochemical mechanism that appears to aid glycan-glycan slippage, whereas the enzymes with better defined biochemical actions, e.g. endoglucanases, do not appear capable of catalyzing wall extension in vitro by themselves (27). This result makes sense in the model shown in Figure 2, where backbone cleavage of matrix polymers would not directly lead to wall extension because it is the lateral, noncovalent bonding between adjacent polysaccharides that is primarily responsible for wall strength. In contrast, the result is difficult to rationalize in the model represented in Figure 1, where breakage of the tethers ought to lead to wall extension.

WALL SYNTHESIS Synthesis, secretion, and integration of new wall polymers is, of course, necessary to maintain the structural integrity of the extending wall in the long term. Currently, there is no evidence that newly secreted polysaccharides directly contribute either to wall loosening or to wall extension. Thus, it is simpler at this stage to think of secretion and integration of wall polymers as separable from wall loosening and extension. This view is supported by the fact that sustained wall extension can indeed occur in vitro, without the direct need for wall synthesis (22, 94). However, it might be that the crucial experiments have not yet been tried to test whether wall extension in vitro can be induced by addition of polysaccharides, perhaps in the presence of enzymes that can assist their integration into the wall, e.g. xyloglucan endotransglycosylase.

Time Scales for Growth Control

It may be important to distinguish between short-term and long-term control of wall extension properties, as these likely involve different control mechanisms. We know that cell wall expansion can start and stop quickly (on the order of one

minute or less), without substantial changes in the structure or composition of the wall. Notable examples include blue-light suppression of hypocotyl elongation (1), growth inhibition by metabolic inhibitors (93), and stimulation of growth by exogenous auxin or fusicoccin (20, 46). In this short time frame, these stimuli have little or no detectable effect on the viscoelastic properties or the composition of the wall, yet they nevertheless modulate wall extension behaviors (23). Modulation of wall-loosening agents seems the likeliest mechanism to account for these rapid growth changes. At the other end of the time scale, a growing cell typically attains a peak expansion rate followed by a gradual slowdown that may take hours or days to complete prior to maturation. During this time, the composition and structure of the wall may change substantially, as a result of secretion of new wall polymers and breakdown, turnover, crosslinking or other modifications of the polymers in muro. In this long time scale, walls become less extensible, as measured by various viscoelastic methods, e.g. (64). Thus there is reason to believe that different processes may contribute to rapid changes versus gradual changes in cell expansion, and that these two types of controls may function simultaneously.

In the sections that follow, I briefly summarize the major candidates for wall-loosening action.

EXPANSINS

An overview of expansin was recently published (25). These proteins were first isolated in 1992 as the mediators of "acid growth." Acid growth refers to the increase in growth rate that occurs when plant cells are placed in acidic solutions (95). Such growth stimulation comes about because the cell wall becomes more extensible at acidic pH. This effect on extensibility is not a direct pH effect on the wall polymers, e.g. on pH-sensitive bonding between wall polysaccharides (116). Instead, it is mediated by one or more protein factors that somehow catalyze wall extension. This conclusion is based on in vitro studies of walls that were treated so as to denature wall proteins (22). Denaturation caused wall extension properties to become insensitive to pH, at least as detected by wall creep and wall stress relaxation assays in the range of pH 4.5 to 7. However, pH sensitivity was largely restored by addition of purified expansins. The pH dependence of expansin-induced wall extension matches closely that of the acid-growth process, with an optimum at pH < 4, and a gradual decline in activity between pH 4 and 7. Expansin activity is also stimulated and inhibited by the same chemical agents that affect acid growth of walls (76). Hence, expansins appear to be the principal protein mediators of acid growth, at least in cucumber hypocotyl walls, where the most detailed work on expansins has been carried out.

Expansins were first defined in terms of their unique action on the rheological behavior of isolated cell walls: They induced long-term pH-dependent extension, and they enhanced stress relaxation of isolated walls over a broad time range, also in a pH-dependent manner (24). This functional definition of expansin has proved robust so far, inasmuch as the only proteins found to possess these properties are clearly homologous to the first expansins purified from cucumber hypocotyls. Since expansins were first cloned (102), many homologous sequences have been identified from a variety of dicots (8, 16, 78, 90, 96, 99, 103), from grasses (18), and even from pine (GenBank Accession No. U65981). Most of these expansins are known only from sequence, not from their biochemical or biological activities. It remains to be seen whether the functional definition of expansin and the sequence definition are fully congruent with each other, i.e. do all those expansin sequences encode proteins with expansin-like rheological effects on walls? The expression pattern of some expansins suggests that these proteins may serve additional functions besides growth, e.g. in fruit softening and cell separation (30, 99). Perhaps these expansins have variant effects on wall rheology. Direct characterization of protein properties are needed to test these possibilities.

Two families of expansins are now recognized, which we are calling α- and β-expansins (25, 26). The two types of expansin have only ~25% amino acid identity to each other, but they appear to be homologous along the full length of their peptide backbones and they have very similar effects on cell wall rheology. However, they may be selective for different wall polymers and their binding properties to walls may differ. Our knowledge of how expansins make the wall more extensible is still limited, but most evidence points to a subtle mechanism, such as a destabilization of glycan-glycan interactions, rather than hydrolysis of matrix polymers.

α-Expansins

Most of what we know about the biochemical action of these proteins comes from studies of native α-expansins extracted from cucumber hypocotyls (75, 76), and the following summary is based primarily on this work. Limited characterization of expansin proteins in tomato leaves (62), maize roots (122), oat coleoptiles (67), soybean hypocotyls (66), and rice internodes (17) has also been published. It should be borne in mind that expansins may have additional roles besides their postulated function in cell growth and the properties of divergent α-expansins may differ from those described here.

Primary transcripts of α-expansins are predicted to encode a protein of ~28 kD, which includes a secretory signal peptide (typically ~23 amino acids) that is removed to make a mature protein of ~25 kD. α-Expansins typically lack motifs for N-linked glycosylation, and biochemical tests indicate that

glycosylation is indeed lacking both in the two α-expansins from cucumber hypocotyls (DJ Cosgrove & DM Durachko, unpublished data) and in two α-expansins from rice internodes (H-T Cho & H Kende, personal communication).

BINDING TO THE WALL In terms of abundance, α-expansins are very minor components of the hypocotyl wall (we estimate ~2 parts protein to 10,000 parts wall, on a dry-weight basis). The protein binds tightly to walls, apparently to noncrystalline surface regions of cellulose (75). Curiously, xyloglucan binding to cellulose neither augmented nor interfered with the capacity of cellulose to bind α-expansin. This observation, together with the finding that expansin binding and expansin activity showed parallel concentration dependencies, led us to suggest that α-expansin action in wall loosening might not involve xyloglucans (75). This inference is weakened, however, by the more recent finding that Zea m1, the maize pollen β-expansin, does not bind strongly to walls, yet it does have robust expansin activity (26). This result shows that tight binding is not essential for expansin activity. It is possible, although not demonstrated, that expansin binding and wall-loosening activity are separable functions, analogous to the separable binding and catalytic activities of many microbial cellulases (50, 115).

What, then, is the significance of expansin binding to the wall? Binding, or lack of it, may be crucial for determining whether expansin protein secreted by one cell can diffuse to adjacent cells and influence their wall properties. For a process such as cell growth, which needs to be regulated in a cell-specific manner, it may be vital to limit expansin diffusion to neighboring cells. In contrast, if the aim is to secrete a protein that will loosen the walls of neighboring cells (e.g. as a pollen tube penetrates stigma and style), then lack of tight wall binding may be important for proper protein function.

It is informative to compare the binding properties of α-expansin with that of another well-studied protein that binds to the surface of cellulose, namely the cellulose-binding domain (CBD) of microbial cellulases. In one study of a CBD from *Clostridium cellulovorans* (47), CBD adsorption to cellulose had an apparent *Kd* of about 1 μM and a maximum binding at saturation of about 1 μmol of CBD per g of cellulose. Comparison with expansin binding can only be approximate at this time because the two studies used different forms of cellulose; nevertheless, the general conclusions from the comparison are probably sound. With α-cellulose from cucumber hypocotyls (75), expansin binding was saturated at a protein:cellulose ratio of about 1:1100. This corresponds to a value of ~40 nmol of expansin per g of cellulose, i.e. about 1/25th the density of CBD binding sites reported above. This low density of expansin binding does not begin to approach full coverage of the cellulose. Evidently, expansins bind to some specific and not very common feature of cellulose or

to a minor contaminant of the α-cellulose fraction that was not extracted by strong base. What this binding site may be is unclear, but spectroscopic studies suggest that cellulose microfibril structure is more complex than commonly pictured (4, 118). The study on expansin binding did not report a *Kd*, but by the same kind of analysis used for the CBD study (47) the expansin *Kd* may be estimated from the data presented to be ~6 nM, that is, about 166 times lower *Kd* than that of the CBD reported above. Thus, the α-expansin from cucumber hypocotyls appears to have much higher affinity and (probably) much lower binding at saturation than the *Clostridium* CBD.

As an aside, the *Clostridium* CBD was reported to have significant effects on growth of roots, root hairs, and pollen tubes (105); also biomass production by transgenic tobacco and poplar plants was enhanced when the CBD gene was expressed ectopically (104). The exact mechanism for these effects is unclear but might result from alteration of cellulose microfibril structure. Unlike the case for expansin, CBD and xyloglucan did compete for the same binding sites on the surface of cellulose. Presumably, CBD and expansin would occupy different sites in the wall, and synergistic effects on wall structure and rheology might result if both were applied simultaneously. I have tested this and other CBDs for expansin-like activity in wall extension and stress relaxation assays but failed to detect any activity (DJ Cosgrove, unpublished results).

There is no indication of cooperativity in binding of α-expansin to cellulose. Likewise, there is little or no cooperativity obvious in the extension-inducing action of these expansins. Hill coefficients were calculated from the dependence of wall extension rates on the concentration of applied expansin. Calculated values varied from ~1.2 to 1.8 in different experiments, indicating little cooperativity (DJ Cosgrove, unpublished data).

MECHANISM OF ACTION Studies to date indicate that α-expansin lacks significant hydrolytic activity against the major polysaccharides of the wall (75, 76). Confirmatory of this conclusion, α-expansin does not lead to a progressive, time-dependent weakening of the wall, as would be expected of a hydrolytic enzyme. Xyloglucan endotransglycosylase activity was not detected in purified expansin fractions (77). On the other hand, expansin can weaken pure cellulose papers (74) and enhance breakdown of cellulose by cellulases (28). The simplest interpretation of these results is that expansin weakens glucan-glucan binding. However, further experimental evidence in support of this hypothesis is needed.

Recent analysis of expansin genes suggests that they encode three functional domains, separated by introns (Figure 3). The N terminus of the primary transcript has a signal peptide of ~22–25 amino acids. The C terminus has a series of conserved tryptophans with spacing similar to that of some bacterial CBDs

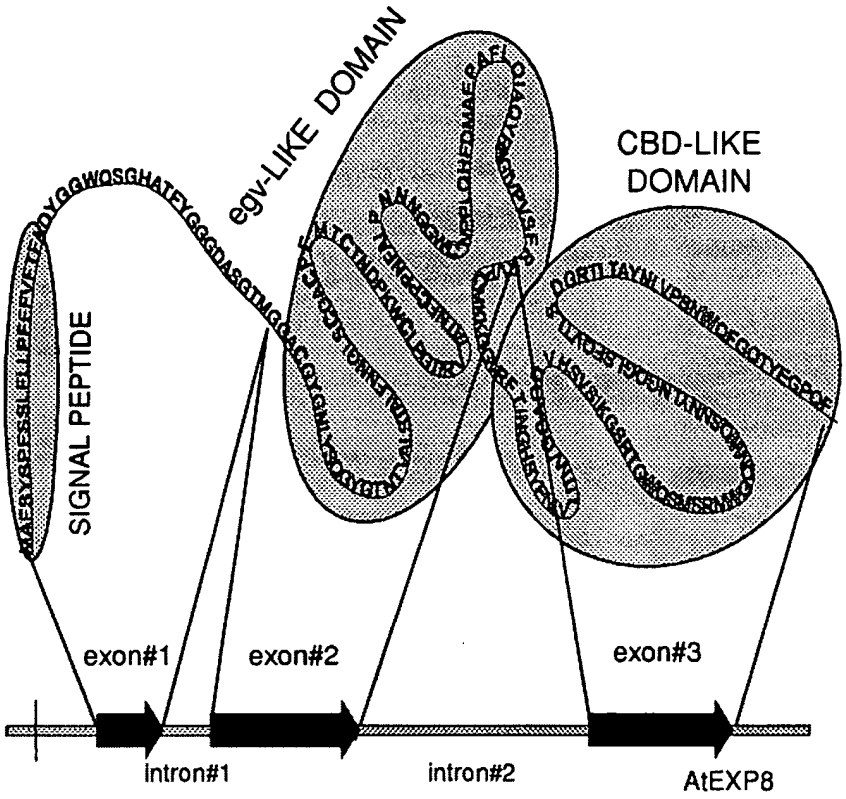

Figure 3 Structure of the expansin gene and protein. The Arabidopsis EXPANSIN8 (AtEXP8) gene contains three exons that encode three potential domains of the protein, including the signal peptide and the N terminus of the mature protein (exon#1), the endoglucanase-like core region (exon#2), and a domain (encoded by exon#3) with some structural resemblance to microbial cellulose-binding domains (CBD).

(35). Tryptophans and related aromatic amino acids are known to be important for protein-carbohydrate interactions; thus the conserved tryptophans and phenylalanines are prime suspects for expansin binding to walls. Although expansin's C terminus thus may function analogously to CBDs, there is no significant sequence homology between the two sequences.

The central part of the protein shows limited, but significant, sequence similarity to an unusual endo-1,4-β-glucanase cloned from *Trichoderma* (100). This endoglucanase, egl5, has been classified as a member of family-45 of glycosidases (52). The protein structure has been solved for the catalytic core of another endoglucanase ("cellulase") member of this family-45 (34). It is

intriguing that what is conserved between the central part of expansin and the catalytic core of family-45 enzymes includes the cysteines and the key residues that make up the putative catalytic site of the endoglucanase. This suggests that the central region of expansin is homologous to the catalytic core of the family-45 endoglucanases. Following up on this molecular hint, we have found that some, but not all, expansins have a barely detectable level of endoglucanase activity when tested with barley 1,3:1,4-β-glucan (28). The endoglucanase activity, *per se*, cannot explain the rheological effect of expansins, because when family-45 endoglucanases were tested they had very high enzyme activity but did not induce wall extension. Thus, these endoglucanases are not expansins. Nevertheless, this sequence relatedness suggests homology and similarity of function, but perhaps using a different substrate or catalyzing a related reaction, such as a transglycosylation. Alternatively, the substrate distortion mechanism proposed for this class of endoglucanase (33, 34) may be conserved and modified in expansins, causing rheological effects, without hydrolytic activity. The search for expansin's mechanism of action goes on....

EFFECTS ON LIVING CELLS In addition to its effects on isolated cell walls, α-expansins have been shown to stimulate enlargement of living cells. All the work along these lines has been done using α-expansin extracted from cucumber hypocotyls. When α-expansin was applied to excised Arabidopsis hypocotyls, it significantly increased elongation (~50% elongation with expansin versus 14% elongation in the controls without expansin) (79). This growth effect was similar to that induced by 1 μM auxin. When α-expansin was applied to cucumber root hairs, it caused swelling and bursting (79). When applied via beads to incipient primordia on excised tomato shoot meristems, expansin caused distortions of the meristem and premature outgrowth of the primordium, at least in a small number of cases (40). When applied to tobacco suspension culture cells, α-expansins induced a threefold stimulation of the rate of cell enlargement (68). Thus it is clear that α-expansins from cucumber hypocotyls can stimulate cell expansion in several heterologous systems.

GENE EXPRESSION Several studies of α-expansin gene expression have been published in the past two years. In rice, four α-expansins were studied by Cho & Kende (18, 19). Two α-expansin proteins were extracted from rice internodes and demonstrated to have classical expansin activity, e.g. they induced wall extension at acidic pH (17). The genes encoding these proteins are expressed in various organs of the rice plant and are induced differentially by treatments that stimulate growth, e.g. gibberellin and submergence (18, 19). In cotton, two studies have identified an α-expansin gene that is highly expressed during the period of maximal growth of the fiber (90, 103). In tomato there

are different α-expansins expressed in different parts of the plant (8, 16, 96). A similar situation holds for expansin expression in Arabidopsis (29, 30). At the time of writing, nine different α-expansin genes have been identified in tomato, six in tobacco, and 22 in Arabidopsis (see the expansin web site at www.bio.psu.edu/expansins/ for an up-to-date census of published expansin sequences and related information). I currently estimate that Arabidopsis has ~30 expansin genes; the exact count will be known when the genome sequencing is completed in the year 2000. Other plant species are likely to have at least this number.

An in situ hybridization study of the tomato shoot apical meristem indicates that an expansin gene is locally expressed in the cells underlying the site of future outgrowth of a leaf primordium (96). This result, in combination with the finding that local expansin application could cause premature primordium outgrowth and an alteration of subsequent phyllotaxy (40), was interpreted to support a variation of Green's model of the meristem (45). In this model, the pattern for leaf primordium initiation is postulated to depend on the pattern of physical forces in the epidermal cell layer, which in turn depends on the geometry of the meristem, the locations of existing local outgrowths, and the physical properties of the epidermal wall. Given this model, it is significant that expansin is not expressed in the epidermal layer of the incipient primordium, but rather in the underlying cells. This suggests that the outer epidermal wall is not loosened at the onset of primordium outgrowth, but rather that it is placed under increased local stress by expansin-induced wall loosening and growth of the underlying cells. Such loosening would tend to cause a displacement of the turgor force of the underlying cells to the restraining epidermal wall, thus increasing its tension. If Green's hypothesis is correct, cells respond to such physical forces by activating appropriate genes for subsequent differentiation processes. Reinhardt et al (96) show that an expansin gene is turned on in subepidermal cells (not in the epidermal cells) prior to primordium emergence; this appears to be the earliest marker of leaf initiation. What initiates expansin gene expression in these cells? Do they participate in the pattern of epidermal stress and strain envisioned in Green's model? If not, then either this earliest sign of primordium initiation is independent of epidermal tensions, or the epidermal cells somehow communicate their status to the underlying cells.

β-Expansins

Unlike α-expansins, which have a high degree of sequence relatedness (~75% amino acid identity, and up to 90% similarity), β-expansins—as we understand them today—are more divergent in sequence and perhaps in function. β-Expansins share only ~25% amino acid identity with α-expansins, but they appear to be homologous to α-expansin in structure (26). Only one member of

this family of expansins has been analyzed for its action on the cell wall, and the properties of this protein, Zea m1, may not be typical of the whole family.

GROUP-1 GRASS POLLEN ALLERGENS Zea m1 is a member of a group of pollen proteins studied for years by immunologists because they (the proteins, not the immunologists) cause hay fever and seasonal asthma in humans. Known as the group-1 grass pollen allergens, the proteins are copiously secreted when grass pollen becomes hydrated. These proteins are highly soluble and are readily washed off of cell walls. Thus, in terms of abundance, solubility, and binding, Zea m1 greatly differs from α-expansins characterized in growing tissues (26). Zea m1 has similar rheological effects on cell walls as cucumber α-expansins, but it shows high specificity for grass cell walls over dicot walls. This suggests that it selectively acts on one of the matrix polymers specific to grasses, e.g. glucuronoarabinoxylan or mixed-linked 1,3:1:4-β-glucan. In contrast, α-expansins (whether of dicot or grass origin) are more active on dicot walls than on grass walls (17, 67, 76). Structural studies of other group-1 allergens have shown that they are glycosylated and two of the prolines near the N terminus are hydroxylated (55, 91). Such posttranslational modifications are not apparent in α-expansins. Zea m1 and its homologs in other grass species probably have a specialized function in pollination, namely to soften the walls of the stigma and style, thereby aiding pollen tube penetration of these tissues (26).

OTHER β-EXPANSINS Although Zea m1 is the only β-expansin directly analyzed for its effects on walls, a search of GenBank reveals other related sequences that likely function as wall-loosening agents (26). First, there are a series of partial cDNAs in the rice EST database. We have sequenced some of these and confirmed their relatedness to the group-1 allergens. The proteins predicted from these cDNAs contain all of the structural hallmarks that are conserved between Zea m1 and α-expansins. Thus, they too may function as wall-loosening agents. What is particularly intriguing is that there are over 50 entries for this class of protein in the rice EST database, and these entries fall into 10 sequence classes (that is, they are derived from 10 different genes). Most of these sequences are from vegetative tissues, so we surmise they have wall-loosening functions that operate in parallel with α-expansins. It is also interesting to note that only a single Arabidopsis EST appears to be a β-expansin. The sequence data to date indicate that there are many more α-expansin genes than β-expansin genes in Arabidopsis, whereas in rice the numbers are more even. This hints that the evolution of the grass cell wall, with its peculiar composition of matrix polymers, went hand-in-hand with the duplication and divergence of the β-expansin family in the grasses. Much work remains to explore the functions of these numerous β-expansins in grasses.

β-Expansins from dicots are rare in GenBank, probably because dicots have few β-expansin genes. A cDNA known as CIM1 (for cytokinin-induced message-1) was originally identified in soybean cell cultures following depletion and return of cytokinin to the growth medium (32). The protein encoded by this message has all the hallmarks of a β-expansin, and its expression pattern is consistent with a role in wall loosening during cytokinin-induced cell proliferation and growth. One unusual feature of CIM1 is that the cytokinin effect on message abundance is regulated, at least in part, by posttranscriptional controls (37). A single Arabidopsis EST falls into the β-expansin family, and there are other sequences that share some remote relatedness to expansins [see discussion in (78)], though most of these remote sequences lack one or more of the key sequence features we currently recognize as diagnostic for expansins: (*a*) a signal peptide; (*b*) a set of conserved cysteines in the N-terminal half of the protein; (*c*) a conserved HFD (his/phe/asp) motif in the middle of the protein, and (*d*) a conserved set of tryptophans at the carboxy-terminal half of the protein (25).

POLYSACCHARIDE HYDROLASES

Cell walls contain numerous enzymes capable of hydrolyzing the major components of the wall matrix, and it is attractive to think that some of these enzymes might function to loosen the wall by breaking load-bearing links between cellulose microfibrils, thereby allowing the wall to extend (9, 42, 57, 85). Alternatively, wall hydrolytic enzymes might stimulate wall extension indirectly: By reducing the size and viscosity of matrix polymers, such enzymes could act synergistically to enhance the action of primary wall-loosening agents, such as expansin. A third alternative is that such hydrolytic enzymes have functions unrelated to wall loosening, e.g. in defense, in signaling, or in polysaccharide processing or breakdown to serve the cell's other metabolic or energy needs. Fry (42) identifies other possible functions for wall hydrolytic enzymes.

Given the model of the wall shown in Figure 1, it seems logical that hydrolysis of the matrix should lead to wall extension, but direct experiments have not shown this to occur. Several hydrolytic enzyme preparations active against cell walls were tested for their ability to directly induce extension of walls in vitro, in much the same way that expansins were demonstrated to act (27). These treatments either failed to cause any effect on wall extension, or they caused the wall to break without an intermediate period of prolonged extension. Thus, they weakened the wall but did not loosen it in the sense of permitting controlled wall yielding and expansion. Some of these enzyme preparations are very potent in hydrolyzing the major structural polysaccharides of the matrix. The *Trichoderma* cellulase preparation used in this study

is probably most active on xyloglucan, which is commonly believed to tether microfibrils together (13, 41, 73, 86). It is therefore surprising, and I think a significant finding, that this and other cellulases did not induce wall extension. It can be argued, with some merit, that plant-derived enzymes have different properties from analogous enzymes obtained from *Trichoderma* or other microbial sources. However, there is no evidence that plant-derived endoglucanases are any more capable of inducing wall extension in vitro than their fungal or bacterial counterparts. Also, the fact that matrix hydrolysis by these enzymes does not cause significant wall extension must cast doubt on the hypothesis that matrix polysaccharide hydrolysis provides a reasonable basis for wall extension and similarly casts in doubt the structural significance of hypothetical glucan tethers between microfibrils, as illustrated in Figure 1.

This argument does not mean that wall hydrolytic activities are irrelevant for wall extension, however, because walls that were hydrolyzed for only a brief time were more sensitive to subsequent expansin-mediated extension (27). Notably, pectinase treatments were at least as effective in enhancing wall extension by expansin as were endoglucanase treatments. These results are more consistent with model B of the cell wall (Figure 2). Thus we might distinguish between two types of wall loosening: primary loosening agents, which are able directly to catalyze wall extension by themselves; and secondary loosening agents, which change the structure of the wall, making it more sensitive to the action of primary loosening agents.

Of the numerous hydrolytic activities in the wall, most growth-related studies have focused on enzymes capable of breaking down matrix glucans, specifically xyloglucan and the mixed-link 1,3:1,4-β-glucan, which are abundant in the growing cell walls of dicots and grass seedlings, respectively.

Endoglucanases

Endoglucanases can hydrolyze glucosidic bonds at a site in the middle of the glucan. Microbial endoglucanases that can attack cellulose typically have long clefts in which single glucan chains can fit so they are precisely placed for catalytic attack by highly conserved residues in the catalytic site (36, 111). A long cleft and a conservation of catalytic amino acids has similarly been observed in a family of plant glucanases that degrade fungal 1,3-β-glucans and grass wall 1,3:1,4-β-glucans (56). No structure has yet been determined for plant-derived glucanases that can degrade β-glucans with a pure 1,4-linked backbone (e.g. cellulose and xyloglucan).

Numerous studies lend circumstantial support to the idea that endoglucanases may be involved in cell expansion. One of the earliest results on this theme was the 1966 report of stimulation of "cellulase" (endoglucanase) activity by high doses of auxin (38), and since then the topic of auxin-induced turnover of matrix

glucans has attracted considerable attention. Several reviews deal with this topic in some detail (48, 60, 70, 98); here I focus mostly on recent contributions to this subject. To summarize briefly the older evidence: (*a*) auxin-induced growth of excised tissues is associated with an enhanced turnover of xyloglucans in dicots and mixed-link glucan in grasses; (*b*) mixed-linked glucans are synthesized and then degraded during the growth of grass coleoptiles and leaves; (*c*) antibodies directed against wall glucanases inhibit auxin-induced growth; and (*d*) lectins and antibodies that bind matrix glucans reduce wall autolysis and inhibit auxin-induced growth. I separate the discussion into work on grasses and dicots.

GRASSES In young grass coleoptiles, the major matrix hemicelluloses include a mixed-link 1,3:1,4-β-glucan, which in the plant world is unique to grasses, and a branched xylan with arabinose and glucuronic acid branches (glucuronoarabinoxylan, or GAX) (12). Both polymers apparently bind to cellulose and could conceivably link cellulose together by binding to more than one microfibril. However, the exact structural role of these hemicelluloses is uncertain, and it has been proposed that the mixed-link glucan might deter wall crosslinking, e.g. as a filler, rather than function as a crosslinking polymer (69). This would account for the fact that this polymer is present only transiently in the growing coleoptile wall and is broken down and resorbed as the coleoptile ceases elongation. The branched xylan (GAX) appears to become debranched as the coleoptile ceases growth (11). Such debranching would likely make GAX adhere to the surface of cellulose and to other xylans more tightly, and this might be a mechanism for reducing wall extensibility. Very little is known about the endogenous enzymes that modify GAX, although enzymes from microbial sources have been characterized.

Recent plant growth work has focused on the enzymes mediating breakdown of mixed-link glucan in maize coleoptiles. The cloning of a cDNA for an unusual endoglucanase from maize coleoptiles was recently reported (112). This enzyme was previously characterized by Hatfield & Nevins (69), who found that it had high specificity for 1,3:1,4-β-glucans and that it appeared to cut the glucan at regular but infrequent spacings, chopping the glucan into 10–15-kD fragments. The cut sites may be places on the polymer consisting of more than five adjacent β-1,4 linkages. The enzyme has a pH optimum of 5 and activity decreases significantly at lower pH, thus the pH dependence does not match the acid growth phenomenon very closely. The reported cDNA sequence encodes a novel protein with a signal peptide of 2.1 kD and a mature polypeptide of 31 kD. This sequence does not fit into any of the 64 families of recognized glycosidases (51), so it is truly novel as an endoglucanase.

This endoglucanase is hypothesized to mediate wall loosening and cell expansion. However, the pattern of its activity raises some doubts on this point.

For example, endoglucanase activity reached maximum levels after coleoptile growth ceased, rather than at the time of maximal extension rate (61).

Although auxin stimulated wall autolysis and growth in excised coleoptiles, it did not increase the amount of the endoglucanase, as detected by immunoblots (61). To account for this discrepancy, Inouhe & Nevins (59) report that a non-enzymatic protein of ~40 kD, which they call an acid wall protein, enhances the activity of maize glucanases (both exo- and endo-type enzymatic activities). Treatment of excised coleoptiles with antibodies directed against the acid wall protein inhibited auxin-induced growth, inferring a role for this protein in growth control. However, the exact mechanism of action of this protein is uncertain; Inouhe & Nevins speculate that auxin might enhance its activity or stimulate its release from sequestration sites in the wall.

Perhaps the strongest argument in favor of wall-loosening activity of glucanases has been from experiments in which specific antibodies against these proteins were found to inhibit auxin-induced growth (e.g. 58). A recent study shows that antibodies against an endo-1,3-β-glucanase can similarly inhibit auxin-induced growth (80). Here's the rub: this study was carried out with mung bean hypocotyls, which are not known to contain 1,3-β-glucans as part of the load-bearing wall. Thus, the action of these antibodies in reducing auxin-induced growth is unlikely to be via direct interference with wall loosening. Instead, they may interfere with communication and translocation through cell walls (120). If this is the case for these mung bean experiments, might it not also hold for the similar experiments with glucanases in maize coleoptiles?

DICOTS In dicots, endo-1,4-β-glucanases (EGs) from several species have been studied; for reviews, see (9, 63). Xyloglucan is the likeliest target for these enzymes, although it is possible that they could hydrolyze some surface 1,4-β-glucan chains that are in noncrystalline regions of the cellulose microfibril (48, 49). Cellulose is relatively stable in growing cells and exhibits negligible turnover, whereas xyloglucan turnover is significant (65, 88), although probably smaller than that of mixed-link glucan in the grasses.

In dicots EGs have been studied in the context of abscission, fruit softening, and auxin-induced growth (9, 97). In tomato, EGs comprise a multigene family of at least seven genes that are expressed in different organs and under the control of different stimuli (6). These genes are all classified in the same family of endoglucases (type E). One member of the tomato family, called *Cel4*, was found to be selectively expressed in the growing region of the tomato hypocotyl, and its message level was increased slightly (~twofold) when etiolated seedlings were treated with high doses of ethylene or auxin (2,4-D), causing lateral swelling of the hypocotyl. A second tomato EG, *Cel7*, was found to be expressed at low levels in both growing and nongrowing regions of the tomato hypocotyl, but

was induced to high levels in both growing and nongrowing hypocotyl regions upon treatment with high doses of auxin (2,4-D) (15). In pea epicotyls and in poplar cell suspension cultures, related auxin-induced EGs were cloned and partially characterized (82, 121). These EG genes encode ~45–50-kD proteins with secretion signal peptides and without motifs for N-linked glycosylation.

Do these EGs function in wall loosening? The time course for induction of EGs by auxin shows that it requires many hours before EG mRNAs accumulate (15), whereas auxin stimulates growth after a lag of ~15 min. This timing argues against a direct role of EGs in the initial phases of auxin-induced growth. Catala et al (15) suggest that aside from a possible wall-loosening role, EGs may function to provide additional acceptor molecules for xyloglucan transglycosylation reactions (see below) or to generate oligosaccharides that may act as signals to modulate further growth (2, 31).

In a recent study, a potent microbial endo-1,4-β-glucanase was expressed in the cell walls of transgenic Arabidopsis (MT Ziegler, SR Thomas, KJ Danna, personal communication). Despite high levels of active enzyme in the cell walls, the plants grew normally and did not show obvious signs of growth disruption. This is a remarkable result because one would expect a significant weakening of the growing wall, if xyloglucans served as direct tethers to connect cellulose microfibrils, as depicted in some current models of the wall (see Figure 1). Either the xyloglucans are somehow protected from the action of the foreign enzyme, or the cell wall is able to compensate for the wholesale wall loosening this enzyme would be expected to inflict on the growing wall. Alternatively, this model may be missing important structural aspects of the wall that determine wall strength and wall growth.

In a similar study, transgenic tobacco plants expressing high levels of microbial xylanases in their walls grew normally, with little evidence of cell wall disturbance (53). Tobacco, as well as other dicots, have xylans in their walls, and it is curious that these potent enzymes did not have substantial phenotypic effects.

The dicot EGs are known principally from their nucleotide sequences, and only limited enzymatic characterization has been reported (119). To evaluate their possible function in wall loosening, it is important to know their substrate specificity, pH dependence, and wall-binding properties, as well as to make direct tests of their ability to induce cell wall extension in living tissues and isolated walls. Hayashi and colleagues have partially characterized EGs from pea epicotyls and poplar cell cultures (49, 81). These enzymes are able to bind cell walls, although binding properties have not been explored in detail. Because their pH optimum is ~6.5, they are unlikely to mediate acid growth. These enzymes have not been tested for their ability to alter cell wall extension properties by direct experiments.

New evidence in support of EG involvement in cell enlargement comes from cloning of the Arabidopsis *KORRIGAN* (*KOR*) gene (83). A plant with a mutation in this gene was found in a screen for short hypocotyl mutants, and it turned out that the growth of most organs was stunted in this mutant. Cytological examination showed that the *kor* mutants had irregular and disturbed patterns of cell expansion. Cell walls were thicker than wild-type walls and appeared disordered. Cloning of KOR showed it to encode an EG, related to the EGs described above, except that it contains a membrane anchor domain. An ortholog of this gene, called Cel3, was recently reported in tomato (7). Cell fractionations indicated that the KOR protein, as well as Cel3, was associated with the plasma membrane. Application of growth hormones had little effect on *KOR* or Cel3 expression. This report shows that *KOR* expression is required for normal cell enlargement, but the exact cellular and biochemical functions of KOR are unclear. If it is indeed anchored to the plasma membrane, then a traditional wall-loosening function is doubtful because it would not have access to most of the cell wall network. Because the newly secreted wall polymers that are closest to the plasma membrane are deposited in a relaxed state, they do not bear the mechanical stress generated by cell turgor and therefore can have little structural influence on cell expansion until the wall extends and they begin to take up some of the wall stress. At this point, they would likely be too far from the membrane-anchored KOR protein to interact with it. Nicol & Hofte (84) suggest several other possible functions of KOR. One speculative possibility is that KOR is part of the cellulose synthase complex and it functions in microfibril formation. This suggestion is consistent with the plasma membrane location of KOR and the fact that a similar membrane-bound EG is essential for cellulose synthesis in *Agrobacterium tumefaciens* (71).

XYLOGLUCAN ENDOTRANSGLYCOSYLASE (XET)

This enzyme, which has also been called endoxyloglucan transferase or EXGT (85), catalyzes a kind of molecular grafting reaction in which the backbone of a xyloglucan is cleaved and one of the resulting half-chains is added to the nonreducing end of a second xyloglucan chain. XET was proposed to have wall-loosening activity (44), but direct tests of its ability to catalyze wall extension in vitro have not supported this hypothesis (77). Several other possible functions for this enzyme, including incorporation of newly secreted xyloglucan into the wall, as well as rearrangement of the xyloglucan-cellulose network during wall assembly and growth, have been suggested by Nishitani (85, 86) and Fry (42, 77). XET may be responsible for the shifts in xyloglucan size recorded in several studies (87, 108, 109, 113). These shifts in size may have an effect on wall extension properties, but this has not been demonstrated directly.

Plant XET proteins are moderate sized (~33 kD) and, at least in some cases, are N-glycosylated. Glycosylation seems to be important for enzyme activity because when TCH4 (an Arabidopsis XET) was enzymatically deglycosylated it lost 98% of its activity (10). XETs contain a presumptive catalytic domain (containing the sequence: DEID-I/F-EFL) that is homologous with the catalytic domain of several bacterial endo 1,3:1,4-β-glucanases. Mutagenesis of the first glutamate (E) to glutamine in TCH4 resulted in an inactive protein (10). This result supports the functional role of the presumptive catalytic domain.

Plant XETs from different sources vary somewhat in their specificity for catalyzing a transglycosylation versus hydrolysis reaction. The difference is whether a saccharide or water can act as an acceptor for the glucan that is cleaved. For example, the enzyme from azuki bean displays only transglycosylation activity (89), whereas the related enzyme from nasturtium seeds mostly has hydrolytic activity (39), that is, it has endoglucanase activity. Four XETs from Arabidopsis lack hydrolytic activity (P Campbell & J Braam, in preparation). These differences in activity are probably due to slight structural changes in or around the catalytic site.

Recent studies have identified XETs as a subset of a larger family of related proteins, called XRPs, for xyloglucan-related proteins (85). Arabidopsis has more than 16 XRPs, which may have distinct patterns of expression in the plant (3, 85; P Campbell & J Braam, personal communication).

Sulová et al (107) showed that a stable xyloglucan-enzyme intermediate is formed during enzyme catalysis, and more recent work from the same group showed that XET can be readily purified by binding the stable XET-xyloglucan complex to paper, then releasing the enzyme by adding oligosaccharides that can serve as acceptors to complete the reaction, freeing the enzyme from the paper-bound xyloglucan (106).

Most biochemical work with XET activity has used solubilized substrates. To test whether newly synthesized xyloglucans in living cells actually formed hybrid molecules with older xyloglucans, Thompson et al (114) double-labeled xyloglucan in *Rosa* cell cultures with $^{13}C/^{3}H$, and then followed the fate of the newly synthesized xyloglucan by monitoring changes in xyloglucan density using isopycnic centrifugation. They found a gradual shift in the density of the newly synthesized xyloglucan over a period of seven days, consistent with the idea that it was integrated into the pre-existing xyloglucan-cellulose network.

Lastly for this section, transgenic tobacco plants were recently produced with reduced expression of an endogenous XET gene that is normally expressed in the leaf vasculature (54). Little change in phenotype was noted, but xyloglucan from the transgenic plants was increased in molecular size in comparison with wild-type plants.

HYDROXYL RADICALS

Fry (43) has proposed a novel mechanism of wall loosening, in which millimolar L-ascorbate nonenzymatically reduces O_2 to H_2O_2 and Cu^{2+} to Cu^{+}, which then react to form hydroxyl radicals (·OH). Hydroxyl radicals are highly reactive and are able to cause oxidative cleavage of diverse polysaccharides nonspecifically. Thus, if ·OH were selectively released in the wall, it might cut xyloglucan or other potential polymers linking cellulose together. By way of evidence, Fry (43) showed that ascorbate-generated ·OH could indeed reduce the viscosity of xyloglucan solutions and that this scission activity has a pH optimum between 4.5 and 5.5; in addition, several free radical scavengers were effective in inhibiting the cleavage activity. Since ascorbate, H_2O_2, and Cu^{2+} are likely to be found in the wall at concentrations effective for ·OH generation, this powerful oxidant could potentially act to loosen walls, if it were produced very close to appropriate targets. Since H_2O_2 is also thought to function in oxidative cross-linking of wall phenolics substances, resulting in wall stiffening (5, 101), there might be a delicately controlled balance between cleavage and cross-linking activities resulting from H_2O_2 production in the wall. The physiological significance of these ideas remains to be determined.

A SUMMARY MODEL OF WALL EXTENSION

A satisfactory model of cell wall extension requires an adequate picture of the polymers and bonds that confer mechanical strength to the wall and that permit time-dependent extension, that is, creep of the wall network. These higher-order aspects of wall architecture are not well understood. Many current discussions of the wall depict its mechanical strength as due to a cellulose-xyloglucan network (as in Figure 1), whereas the physical properties of the wall, summarized by Preston (92), are more consistent with an assemblage of weak bonds gluing wall polysaccharides together, as in Figure 2. This alternative model is also supported by some wall extraction studies, summarized by Talbott & Ray (110), and by the effects of enzymatic treatments on wall extension behaviors (27).

For the reasons described previously, it may be important to distinguish between primary and secondary wall-loosening agents. I suggest an operational definition of primary loosening agents as those substances and processes that are competent and sufficient to induce extension of walls in vitro. Expansins fit this definition, whereas various wall enzymes with putative wall-loosening functions, such as endo-1,4-β-glucanase and XET, have not been shown to possess such activity. Secondary wall-loosening agents can be defined as those substances and processes that modify wall structure to enhance the action of

primary agents. It is possible, although not actually demonstrated, that plant endoglucanases, XET, pectinases, as well as secretion of specific wall polymers and production of hydroxyl radicals, could function as secondary wall-loosening agents. The activity of primary and secondary wall-loosening agents could be modulated in various ways, e.g. by changes in wall pH, by secretion of ligands that activated or inactivated wall enzymes, by secretion of substrates, etc. Additionally, the wall could be modified by other enzymatic activities that cross-linked the wall or that changed the wall polymers such that they were no longer acted upon by wall-loosening agents. This latter notion suggests a method for assessing wall "stiffening" or "tightening" activities, that is, by testing whether walls pretreated with specific enzymes are reduced in their ability to respond in vitro to primary loosening agents.

Beyond in vitro experiments, it is important to extend the results to living cells and to whole plants and to test our knowledge of how the wall works by genetic manipulations. The limited transgenic experiments carried out so far with wall enzymes have shown that the plant cell wall is amazingly resilient to assault by these transgenic (mostly microbial) enzymes. Genetic engineering, or perhaps better stated, genetic tinkering, holds much potential for defining the cellular machinery needed for wall expansion.

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Literature Cited

1. Addink C, Meijer G. 1972. Kinetic studies on the auxin effect and the influence of cycloheximide and blue light. In *Plant Growth Substances 1970*, ed. DJ Carr, pp. 68–75. Berlin/New York: Springer-Verlag
2. Aldington S, Fry SC. 1993. Oligosaccharins. *Adv. Bot. Res.* 19:1–101
3. Antosiewicz DM, Purugganan MM, Polisensky DH, Braam J. 1997. Cellular localization of Arabidopsis xyloglucan endotransglycosylase-related proteins during development and after wind stimulation. *Plant Physiol.* 115:1319–28
4. Atalla RH, Hackney JM, Uhlin I, Thompson NS. 1993. Hemicelluloses as structure regulators in the aggregation of native cellulose. *Int. J. Biol. Macromol.* 15:109–12
5. Brisson LF, Tenhaken R, Lamb C. 1994. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell* 6:1703–12
6. Brummell DA, Bird CR, Schuch W, Bennett AB. 1997. An endo-1,4-β-glucanase expressed at high levels in rapidly expanding tissues. *Plant Mol. Biol.* 33:87–95
7. Brummell DA, Catala C, Lashbrook CC, Bennett AB. 1997. A membrane-

anchored E-type endo-1,4-β-glucanase is localized on Golgi and plasma membranes of higher plants. *Proc. Natl. Acad. Sci. USA* 94:4794–99
8. Brummell DA, Harpster MH, Dunsmuir P. 1999. Differential expression of expansin gene family members during growth and ripening of tomato fruit. *Plant Mol. Biol.* In press
9. Brummell DA, Lashbrook CC, Bennett AB. 1994. Plant endo-1,4-β-D-glucanases. Structure, properties, and physiological functions. *ACS Symp. Ser. Enzym. Convers. Biomass Fuels Prod.* 566:100–29
10. Campbell P, Braam J. 1998. Co- and/or post-translational modifications are critical for TCH4 XET activity. *Plant J.* 5:553–61
11. Carpita NC. 1984. Cell wall development in maize coleoptiles. *Plant Physiol.* 76:205–12
12. Carpita NC. 1996. Structure and biogenesis of the cell walls of grasses. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:445–76
13. Carpita NC, Gibeaut DM. 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* 3:1–30
14. Cassab GI. 1998. Plant cell wall proteins. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49:281–309
15. Catala C, Rose JKC, Bennett AB. 1997. Auxin regulation and spatial localization of an endo-1,4-β-D-glucanase and a xyloglucan endotransglycosylase in expanding tomato hypocotyls. *Plant J.* 12:417–26
16. Chen F, Bradford KJ. 1998. Expansin genes are expressed in tomato seeds in association with germination. *Annu. Meet. Am. Soc. Plant Physiol. Abstr. 47*
17. Cho HT, Kende H. 1997. Expansins in deepwater rice internodes. *Plant Physiol.* 113:1137–43
18. Cho HT, Kende H. 1997. Expression of expansin genes is correlated with growth in deepwater rice. *Plant Cell* 9:1661–71
19. Cho HT, Kende H. 1998. Tissue localization of expansins in rice. *Plant J.* 15:805–12
20. Cleland RE. 1976. Fusicoccin-induced growth and hydrogen ion excretion of *Avena* coleoptiles: relation to auxin responses. *Planta* 128:201–6
21. Cleland RE, Cosgrove DJ, Tepfer M. 1987. Long-term acid-induced wall extension in an *in vitro* system. *Planta* 170:379–85
22. Cosgrove DJ. 1989. Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls. *Planta* 177:121–30
23. Cosgrove DJ. 1993. Wall extensibility: its nature, measurement, and relationship to plant cell growth. *New Phytol.* 124:1–23
24. Cosgrove DJ. 1997. Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. *Plant Cell* 9:1031–41
25. Cosgrove DJ. 1998. Cell wall loosening by expansins. *Plant Physiol.* 118:333–39
26. Cosgrove DJ, Bedinger PA, Durachko DM. 1997. Group I allergens of grass pollen as cell wall loosening agents. *Proc. Natl. Acad. Sci. USA* 94:6559–64
27. Cosgrove DJ, Durachko DM. 1994. Autolysis and extension of isolated walls from growing cucumber hypocotyls. *J. Exp. Bot.* 45:1711–19
28. Cosgrove DJ, Durachko DM, Li L-C. 1998. Expansins may have cryptic endoglucanase activity and can synergize the breakdown of cellulose by fungal cellulases. *Annu. Meet. Am. Soc. Plant Physiol. Abstr. 171*
29. Cosgrove DJ, Durachko DM, Shcherban TY. 1998. The expansin super family in Arabidopsis. *9th Int. Conf. Arabidopsis Res.* 341 (Abstr.)
30. Cosgrove DJ, Shcherban TY, Durachko DM. 1998. Highly specific and distinct expression patterns for two alpha-expansin genes in Arabidopsis. *9th Int. Conf. Arabidopsis Res.* 166 (Abstr.)
31. Creelman RA, Mullet JE. 1997. Oligosaccharins, brassinolides, and jasmonates: nontraditional regulators of plant growth, development, and gene expression. *Plant Cell* 9:1211–23
32. Crowell DN. 1994. Cytokinin regulation of a soybean pollen allergen gene. *Plant Mol. Biol.* 25:829–35
33. Davies GJ. 1998. Structural studies on cellulases. *Biochem. Soc. Trans.* 26:167–73
34. Davies GJ, Tolley SP, Henrissat B, Hjort C, Schulein M. 1995. Structures of oligosaccharide-bound forms of the endoglucanase V from *Humicola insolens* at 1.9 Å resolution. *Biochemistry* 34:16210–20
35. Din N, Forsythe IJ, Burtnick LD, Gilkes NR, Miller RC, et al. 1994. The cellulose-binding domain of endoglucanase A (CenA) from *Cellulomonas fimi*: evidence for the involvement of tryptophan residues in binding. *Mol. Microbiol.* 11:747–55
36. Divne C, Ståhlberg J, Teeri TT, Jones TA.

1998. High-resolution crystal structures reveal how a cellulose chain is bound in the 50 Å long tunnel of cellobiohydrolase I from *Trichoderma reesei*. *J. Mol. Biol.* 275:309–25
37. Downes BP, Crowell DN. 1998. Cytokinin regulates the expression of a soybean β-expansin gene by a post-transcriptional mechanism. *Plant Mol. Biol.* 37:437–44
38. Fan D-F, Maclachlan GA. 1966. Control of cellulase activity by indolecaectic acid. *Can. J. Bot.* 44:1025–34
39. Fanutti C, Gidley MJ, Reid JSG. 1993. Action of a pure xyloglucan endo-transglycosylase (formerly called xyloglucan-specific endo-1,4-β-D-glucanase) from the cotyledons of germinated nasturtium seeds. *Plant J.* 3:691–700
40. Fleming AJ, McQueen-Mason S, Mandel T, Kuhlemeier C. 1997. Induction of leaf primordia by the cell wall protein expansin. *Science* 276:1415–18
41. Fry SC. 1989. Cellulases, hemicelluloses and auxin-stimulated growth: a possible relationship. *Physiol. Plant.* 75:532–36
42. Fry SC. 1995. Polysaccharide-modifying enzymes in the plant cell wall. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46:497–520
43. Fry SC. 1998. Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals. *Biochem. J.* 332:507–15
44. Fry SC, Smith RC, Renwick KF, Martin DJ, Hodge SK, Matthews KJ. 1992. Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. *Biochem. J.* 282:821–28
45. Green PB. 1997. Expansin and morphology: a role for biophysics. *Trends Plant Sci.* 2:365–66
46. Green PB, Cummins WR. 1974. Growth rate and turgor pressure: auxin effect studied with an automated apparatus for single coleoptiles. *Plant Physiol.* 54:863–69
47. Hamamoto T, Foong F, Shoseyov O, Doi RH. 1992. Analysis of functional domains of endoglucanases from *Clostridium cellulovorans* by gene cloning, nucleotide sequencing and chimeric protein construction. *Mol. Gen. Genet.* 231:472–79
48. Hayashi T. 1989. Xyloglucans in the primary cell wall. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:139–68
49. Hayashi T, Wong YS, Maclachlan G. 1984. Pea xyloglucan and cellulose. II. Hydrolysis by pea endo-1,4-β-glucanases. *Plant Physiol.* 75:605–10
50. Hazlewood GP, Gilbert HJ. 1998. Structure and function analysis of *Pseudomonas* plant cell wall hydrolases. *Biochem. Soc. Trans.* 26:185–90
51. Henrissat B. 1998. Glycosidase families. *Biochem. Soc. Trans.* 26:153–56
52. Henrissat B, Teeri TT, Warren RA. 1998. A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants. *FEBS Lett.* 425:352–54
53. Herbers K, Flint HJ, Sonnewald U. 1996. Apoplastic expression of the xylanase and β (1-3,1-4) glucanase domains of the xynD gene from *Ruminococcus flavefaciens* leads to functional polypeptides in transgenic tobacco plants. *Mol. Breeding* 2:81–87
54. Herbers K, Lorences EP, Barrachina C, Sonnewald U. 1998. Functional characterization of xyloglucan endotransglycosylase in transgenic tobacco: identification of a novel plant defense mechanism. *8th Int. Cell Wall Meet.* 7.11 (Abstr.)
55. Hiller KM, Esch RE, Klapper DG. 1997. Mapping of an allergenically important determinant of grass group I allergens. *J. Allergy Clin. Immunol.* 100:335–40
56. Hoj PB, Fincher GB. 1995. Molecular evolution of plant β-glucan endohydrolases. *Plant J.* 7:367–79
57. Hoson T. 1993. Regulation of polysaccharide breakdown during auxin-induced cell wall loosening. *J. Plant Res.* 103:369–81
58. Hoson T, Masuda Y, Nevins DJ. 1992. Comparison of the outer and inner epidermis. Inhibition of auxin-induced elongation of maize coleoptiles by glucan antibodies. *Plant Physiol.* 98:1298–303
59. Inouhe M, Nevins D. 1997. Regulation of cell wall glucanase activities by non-enzymic proteins in maize coleoptiles. *Int. J. Biol. Macromol.* 21:15–20
60. Inouhe M, Nevins DJ. 1997. Changes in the autolytic activities of maize coleoptile cell walls during coleoptile growth. *Plant Cell Physiol.* 38:161–67
61. Inouhe M, Nevins DJ. 1998. Changes in the activities and polypeptide levels of exo- and endoglucanases in cell walls during developmental growth of *Zea mays* coleoptiles. *Plant Cell Physiol.* 39:762–68
62. Keller E, Cosgrove DJ. 1995. Expansins in growing tomato leaves. *Plant J.* 8:795–802
63. Kemmerer EC, Tucker ML. 1994. Comparative study of cellulases associated with adventitious root initiation, apical buds, and leaf, flower, and pod abscission

zones in soybean. *Plant Physiol.* 104: 557–62
64. Kutschera U. 1996. Cessation of cell elongation in rye coleoptiles is accompanied by a loss of cell-wall plasticity. *J. Exp. Bot.* 47:1387–94
65. Labavitch JM, Ray PM. 1974. Relationship between promotion of xyloglucan metabolism and induction of elongation by IAA. *Plant Physiol.* 54:499–502
66. Li L-C, Wang X-C, Jing J-H. 1998. The existence of expansin and its properties in the hypocotyls of soybean seedlings. *Acta Bot. Sin.* 40:627–34
67. Li Z-C, Durachko DM, Cosgrove DJ. 1993. An oat coleoptile wall protein that induces wall extension in vitro and that is antigenically related to a similar protein from cucumber hypocotyls. *Planta* 191:349–56
68. Link BM, Cosgrove DJ. 1998. Acid growth response and α-expansins in suspension cultures of *Nicotiana tabacum* L. cv. BY2. *Plant Physiol.* 118:907–16
69. Luttenegger DG, Nevins DJ. 1985. Transient nature of a (1->3),(1->4)-β-D-glucan in *Zea mays* coleoptile cell walls. *Plant Physiol.* 77:175–78
70. Maclachlan G. 1988. β-glucanases from *Pisum sativum*. *Methods Enzymol.* 160: 382–91
71. Matthysse AG, White S, Lightfoot R. 1995. Genes required for cellulose synthesis in *Agrobacterium tumefaciens*. *J. Bacteriol.* 177:1069–75
72. McCann MC, Roberts K. 1991. Architecture of the primary cell wall. In *Cytoskeletal Basis of Plant Growth and Form*, ed. C Lloyd, pp. 109–29. London/San Diego: Academic
73. McCann MC, Wells B, Roberts K. 1990. Direct visualization of cross-links in the primary plant cell wall. *J. Cell Sci.* 96:323–34
74. McQueen-Mason S, Cosgrove DJ. 1994. Disruption of hydrogen bonding between wall polymers by proteins that induce plant wall extension. *Proc. Natl. Acad. Sci. USA* 91:6574–78
75. McQueen-Mason S, Cosgrove DJ. 1995. Expansin mode of action on cell walls: analysis of wall hydrolysis, stress relaxation, and binding. *Plant Physiol.* 107:87–100
76. McQueen-Mason S, Durachko DM, Cosgrove DJ. 1992. Two endogenous proteins that induce cell wall expansion in plants. *Plant Cell* 4:1425–33
77. McQueen-Mason S, Fry SC, Durachko DM, Cosgrove DJ. 1993. The relationship between xyloglucan endotransglycosylase and in vitro cell wall extension in cucumber hypocotyls. *Planta* 190:327–31
78. Michael AJ. 1996. A cDNA from pea petals with sequence similarity to pollen allergen, cytokinin-induced and genetic tumour-specific genes: identification of a new family of related sequences. *Plant Mol. Biol.* 30:219–24
79. Moore RC, Fleeker D, Cosgrove DJ. 1995. Expansin action on cells with tip growth and diffuse growth. *J. Cell. Biochem. Suppl.* 21A:457 (Abstr. J5–312)
80. Mutaftschiev S, Prat R, Pierron M, Devilliers G, Goldberg R. 1997. Relationship between cell-wall β-1,3-endglucanase activity and auxin-induced elongation in mung bean hypocotyl segments. *Protoplasma* 199:49–56
81. Nakamura S, Hayashi T. 1993. Purification and properties of an extracellular endo-1,4-β-glucanase from suspension-cultured poplar cells. *Plant Cell Physiol.* 34:1009–13
82. Nakamura S, Mori H, Sakai F, Hayashi T. 1995. Cloning and sequencing of a cDNA for poplar endo-1,4-β-glucanase. *Plant Cell Physiol.* 36:1229–35
83. Nicol F, His I, Jauneau A, Vernhettes S, Canut H, Höfte H. 1998. A plasma membrane-bound putative endo-1,4-β-D-glucanase is required for normal wall assembly and cell elongation in *Arabidopsis*. *EMBO J.* 17:5563–76
84. Nicol F, Hofte H. 1998. Plant cell expansion: scaling the wall. *Curr. Opin. Plant Biol.* 1:12–17
85. Nishitani K. 1997. The role of endoxyloglucan transferase in the organization of plant cell walls. *Int. Rev. Cytol.* 173:157–206
86. Nishitani K. 1998. Construction and restructuring of the cellulose-xyloglucan framework in the apoplast as mediated by the xyloglucan-related protein family—a hypothetical scheme. *J. Plant Res.* 111:1–8
87. Nishitani K, Masuda Y. 1982. Acid pH-induced structural changes in cell wall xyloglucans in *Vigna angularis* epicotyl segments. *Plant Sci. Lett.* 28:87–94
88. Nishitani K, Masuda Y. 1983. Auxin-induced changes in the cell wall xyloglucans: effects of auxin on the two different subfractions of xyloglucans in the epicotyl cell wall of *Vigna angularis*. *Plant Cell Physiol.* 24:345–55
89. Nishitani K, Tominaga T. 1992. Endoxyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule

to another xyloglucan molecule. *J. Biol. Chem.* 267:21058–64
90. Orford SJ, Timmis JN. 1998. Specific expression of an expansin gene during elongation of cotton fibres. *BBA-Gene Struct. Expr.* 1398:342–46
91. Petersen A, Becker WM, Schlaak M. 1993. Characterization of grass group I allergens in timothy grass pollen. *J. Allergy Clin. Immunol.* 92:789–96
92. Preston RD. 1979. Polysaccharide conformation and cell wall function. *Annu. Rev. Plant Physiol.* 30:55–78
93. Ray PM, Ruesink AW. 1962. Kinetic experiments on the nature of the growth mechanism in oat coleoptile cells. *Dev. Biol.* 4:377–97
94. Rayle DL, Cleland RE. 1970. Enhancement of wall loosening and elongation by acid solutions. *Plant Physiol.* 46:250–53
95. Rayle DL, Cleland RE. 1992. The acid growth theory of auxin-induced cell elongation is alive and well. *Plant Physiol.* 99:1271–74
96. Reinhardt D, Wittwer F, Mandel T, Kuhlemeier C. 1998. Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem. *Plant Cell* 10:1427–37
97. Rose JK, Hadfield KA, Labavitch JM, Bennett AB. 1998. Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. *Plant Physiol.* 117:345–61
98. Rose JKC, Brummell DA, Bennett AB. 1996. Two divergent xyloglucan endotransglycosylases exhibit mutually exclusive patterns of expression in nasturtium. *Plant Physiol.* 110:493–99
99. Rose JKC, Lee HH, Bennett AB. 1997. Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proc. Natl. Acad. Sci. USA* 94:5955–60
100. Saloheimo A, Henrissat B, Hoffren AM, Teleman O, Penttilä M. 1994. A novel, small endoglucanase gene, *egl5*, from *Trichoderma reesei* isolated by expression in yeast. *Mol. Microbiol.* 13:219–28
101. Schopfer P. 1996. Hydrogen peroxide-mediated cell-wall stiffening in vitro in maize coleoptiles. *Planta* 199:43–49
102. Shcherban TY, Shi J, Durachko DM, Guiltinan MJ, McQueen-Mason S, et al. 1995. Molecular cloning and sequence analysis of expansins—a highly conserved, multigene family of proteins that mediate cell wall extension in plants. *Proc. Natl. Acad. Sci. USA* 92:9245–49
103. Shimizu Y, Aotsuka S, Hasegawa O, Kawada T, Sakuno T, et al. 1997. Changes in levels of mRNAs for cell wall-related enzymes in growing cotton fiber cells. *Plant Cell Physiol.* 38:375–78
104. Shoseyov O, Shpigel E, Shanil Z, Roiz L. 1998. Cellulose binding domain increases cellulose synthase activity and biomass of transgenic plants. *8th Int. Cell Wall Meet.* 1.55 (Abstr.)
105. Shpigel E, Roiz L, Goren R, Shoseyov O. 1998. Bacterial cellulose-binding domain modulates in vitro elongation of different plant cells. *Plant Physiol.* 117:1185–94
106. Sulová Z, Farkas V. 1998. A method for purification of XET based on affinity sorption of XET: xyloglucan complex on cellulose. *8th Int. Cell Wall Meet.* 7.41 (Abstr.)
107. Sulová Z, Takácová M, Steele NM, Fry SC, Farkas V. 1998. Xyloglucan endotransglycosylase: evidence for the existence of a relatively stable glycosyl-enzyme intermediate. *Biochem. J.* 330: 1475–80
108. Talbott LD, Pickard BG. 1994. Differential changes in size distribution of xyloglucan in the cell walls of gravitropically responding *Pisum sativum* epicotyls. *Plant Physiol.* 106:755–61
109. Talbott LD, Ray PM. 1992. Changes in molecular size of previously deposited and newly synthesized pea cell wall matrix polysaccharides. *Plant Physiol.* 98:369–79
110. Talbott LD, Ray PM. 1992. Molecular size and separability features of pea cell wall polysaccharides. Implications for models of primary wall structure. *Plant Physiol.* 92:357–68
111. Teeri TT, Koivula A, Linder M, Wohlfahrt G, Divne C, Jones TA. 1998. *Trichoderma reesei* cellobiohydrolases: why so efficient on crystalline cellulose? *Biochem. Soc. Trans.* 26:173–78
112. Thomas BR, Simmons C, Inouhe M, Nevins DJ. 1998. Maize coleoptile endoglucanase is encoded by a novel gene family (Accession No. AF072326). PGR 98–143. *Plant Physiol.* 117:1525–25 (Abstr.)
113. Thompson JE, Fry SC. 1997. Trimming and solubilization of xyloglucan after deposition in the walls of cultured rose cells. *J. Exp. Bot.* 48:297–305
114. Thompson JE, Smith RC, Fry SC. 1997. Xyloglucan undergoes interpolymeric transglycosylation during binding to the plant cell wall *in vivo*: evidence from $^{13}C/^{3}H$ dual labelling and isopycnic centrifugation in caesium trifluoroacetate. *Biochem. J.* 327:699–708
115. Tomme P, Boraston A, McLean B, Kormos J, Creagh AL, et al. 1998. Characterization and affinity applications of

cellulose-binding domains. *J. Chromatogr. B* 715:283–96
116. Valent BS, Albersheim P. 1974. The structure of plant cell walls. V. On the binding of xyloglucan to cellulose fibers. *Plant Physiol.* 54:105–8
117. Veytsman BA, Cosgrove DJ. 1998. A model of cell wall expansion based on thermodynamics of polymer networks. *Biophys. J.* 75:2240–50
118. Victor RJ, Ha MA, Aperley DC, Jarvis MC. 1998. Internal structure of cellulose microfibrils in primary cell walls. *8th Int. Cell Wall Meet.* 3.02 (Abstr.)
119. Wong YS, Fincher GB, Maclachlan GA. 1977. Kinetic properties and substrate specificities of two cellulases from auxin-treated pea epicotyls. *J. Biol. Chem.* 252:1402–7
120. Wong YS, Maclachlan GA. 1980. 1,3-β-D-Glucanases from *Pisum sativum* seedlings. III. Development and distribution of endogenous substrates. *Plant Physiol.* 65:222–28
121. Wu S-C, Blumer JM, Darvill AG, Albersheim P. 1996. Characterization of an endo-β-1,4-glucanase gene induced by auxin in elongating pea epicotyls. *Plant Physiol.* 110:163–70
122. Wu Y, Sharp RE, Durachko DM, Cosgrove DJ. 1996. Growth maintenance of the maize primary root at low water potentials involves increases in cell wall extensibility, expansin activity and wall susceptibility to expansins. *Plant Physiol.* 111:765–72
123. Yamamoto R, Sakurai N. 1990. A computer simulation of the creep process of the cell wall using stress relaxation parameters. *Biorheology* 27:759–68